bio-protocol Preprint

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Collection and procession of peripheral blood mononuclear cell, biliary brush samples, and liver tissue

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Updated date: Feb 27, 2023

An abbreviated version of this protocol was published in Science Translational Medicine in Jun 2021

A biliary immune landscape map of primary sclerosing cholangitis reveals a dominant network of neutrophils and tissue-resident T cells

DOI: 10.1126/scitranslmed.abb3107

Detailed protocol

Detailed protocol

The protocol enables the isolation of leukocytes from biliary brush samples (Boson Scientific RX biliary 2.1 mm cytology cytobrush) after endoscopic retrograde cholangiopancreatography (ERCP)

1. Reagents

- RPMI 1640 medium (Thermo Fisher Scientific)
- Fetal calf serum (Thermo Fisher Scientific)
- L-glutamine (Invitrogen)
- DNase (0.2 mg/mL; Roche, Mannheim, Germany)
- Collagenase II (0.25mg/mL, Sigma-Aldrich, St. Louis, Mo)

Incomplete medium: RPMI 1640 medium
Complete medium: RPMI 1640 medium containing 10% FCS and 1 mM L-glutamine

Equipment

- Sterile hood
- 15 ml Falcon tubes
- PTFE Stirrer Bar, Micro, 5x2mm (Cowie Technology Group, Ridgeway, UK)
- Forceps
- Water bath / Magnetic Stirrer System
- Rotina 420R Centrifuge

Procedure

1. Biliary brush samples were collected during ERCP and directly transferred into a 15 ml Falcon tube containing 3-5 ml of complete RPMI 1640 medium for immediate procession.
2. The water bath was pre-warmed to start the enzymatic digestion at precisely 37°C.
3. Under sterile conditions, the cells were transferred with the help of forceps into a new 15 ml Flacon tube containing 1 ml of incomplete RPMI 1640 medium without FCS and L-glutamine.
4. For cellular detachment from the cytobrush, collagenase II (0.25mg/mL) and DNase (0.2 mg/mL) were added into the medium.
5. One magnetic stir bar was added into the tube and the tube was subsequently transferred to the water bath with the magnetic stirrer system on and incubated for 15min at 37°C.
6. To stop the enzymatic digestion, the tube was removed from the water bath and 1 ml of complete RPMI medium was directly added into the 15 ml Falcon tube.
7. To increase cell yield, the cytobush was flushed with complete medium using a 200 ml pipette.
8. All medium/cell containing tubes were pooled, which was followed by a centrifugation at 1500 rpm for 5 min at RT.
9. The supernatants were discarded, and the cells cryopreserved or used for flow cytometry.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Björkström, N. (2023). Collection and procession of peripheral blood mononuclear cell, biliary brush samples, and liver tissue. Bio-protocol Preprint.

bio-protocol.org/prep2152.

2. Zimmer, C. L., Seth, E. V., Buggert, M., Strauss, O., Hertwig, L., Nguyen, S., Wong, A. Y. W., Zotter, C., Berglin, L., Michaëlsson, J., Hansson, M. R., Arnelo, U., Sparrelid, E., Ellis, E. C. S., Söderholm, J. D., Keita, Å. V., Holm, K., Özenci, V., Hov, J. R., Mold, J. E., Cornillet, M., Ponzetta, A., Bergquist, A. and Björkström, N. K.(2021). A biliary immune landscape map of primary sclerosing cholangitis reveals a dominant network of neutrophils and tissue-resident T cells. Science Translational Medicine 13(599). DOI: 10.1126/scitranslmed.abb3107

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